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22 Nanotechnology and Biomaterials

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22.1 INTRODUCTION

Biomaterials have received a considerable amount of attention over the last 30 years as a means of treating diseases and easing suffering. The focus of treatment is no longer a conventional pharmaceutical formulation but rather a combination of device-integrated biomaterial and the necessary therapeutic

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treatment. Biomaterials have found applications in approximately 8000 different kinds of medical devices [1], which have been used in repairing skeletal systems, returning cardiovascular functionality, replacing organs, and repairing or returning senses [2]. Even though biomaterials have had a pronounced impact in medical treatment, a need still exists to be able to design and develop better polymer, ceramic, and metal systems [3].

Polymeric biomaterials originated as off-the-shelf materials that clinicians were able to use in solving a problem, for example, dialysis tubing was originally made of cellulose acetate, vascular grafts were fabricated from Dacron, and artificial hearts were molded from polyurethanes (PUs) [2]. However, these materials did not possess the chemical, physical, and biological properties necessary to prevent further complications. Recent advances in synthetic techniques have allowed these properties to be imparted on polymeric biomaterials, which help to alleviate accompanying biocompatibility issues. Nanotechnology, as this chapter will describe, further adds to the ability of chemically tailoring polymeric materials to provide more opportunities for revolutionary breakthroughs in the science and technology associated with developing novel devices. Undoubtedly, nanoscale science and engineering has the potential to have a profound impact on medical science and technology, which will lead to improved diagnostics and enhanced therapeutic methods [4].

For ceramics and metals, similar advancements through the use of nanotechnology can be envisioned. For example, bioimplants, administered to humans over the past nine decades, have mostly been synthetic prostheses consisting of ceramic or metal particles and grain sizes with conventional dimensions (of the order of 1 to 10^4 μm). But the lack of sufficient bonding of these synthetic implants to surrounding body tissues has, in recent years, led to the investigation of novel material formulations. One such category of materials, nanophase ceramics and metals (or materials with constituent components less than 100 nm in at least one direction), can be used to synthesize implants with surface roughness similar to that of natural tissues. Natural tissues have numerous nanometer features available for cellular interactions since they are composed of many nanostructures (specifically, proteins). Several nanophase ceramic and metal biomedical implants are currently being investigated, and are likely to gain approvals for clinical use in the near future.

One area in which nanophase polymers, ceramics, and metals are being heavily investigated involves orthopedic/dental applications and this is for a good reason. Since 1990, the total number of hip replacements, which is the replacement of the femoral and hip bones, has been steadily increasing [5–11]. In fact, in the United States alone in 2000 there were 152,000 total hip replacements — a 33% increase from the number performed in 1990 and a little over half of the projected number of total hip replacements (272,000) by the year 2030 [5–8]. However, in 1997, 12.8% of the total hip arthroplasties were simply due to revision surgeries of previously implanted failed hip replacements [5–8].

The fact that such a high percentage of hip replacements performed every year are revision surgeries is not surprising when you consider the life expectancy of the implant versus that of the patient receiving the implant. Consistently, over 30% of those requiring total hip replacements have been below the age of 65 and even those at the age of 65 have a life expectancy of 17.9 years [9–11]. Females, who comprise a majority of those receiving total hip replacements, have a life expectancy of 19.2 years at the age of 65. Since the longevity of implants ranges only from about 12 to 15 years, even the majority of those who receive bone implants at the age of 65 will require at least one revision surgery before the end of their lives [9–11].

For the dental community the story is not any better. Since dental implants may be necessary for the young and old alike, it is imperative that they are able to last for the duration of the patient's life. Recent studies have found that dental implants which have been used in over 300,000 cases in the United States, have up to a 96% success rate (meaning that the implant was not mobile and was noninflamed) after 5 years, 80% after 10 years, and less than 75% after 15 years [5–11].

The above information strongly suggests that the longevity of the current prostheses is a reoccurring problem for the orthopedic/dental community that has to be dealt with since current approaches clearly fail. Orthopedic implant failure can be due to numerous reasons, including poor initial bonding of the implant to juxtaposed bone, generation of wear debris that lodges between the

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implant and surrounding bone to cause bone cell death, and stress and strain imbalances between the implant and surrounding bone causing implant loosening and eventual failure [12]. Although there are many reasons why implants fail, a central one is the lack of sufficient bone regeneration around the implant immediately after insertion [12]. Shockingly, about a quarter of dental implant failures (of those that failed between 3 and 6 months) are attributed to incomplete healing of the implant to juxtaposed bone [5–11]. Importantly, this leads to eventual implant loosening and regions for possible wear debris to situate between the implant and surrounding bone, further complicating bone loss [12–15].

Nanotechnology is playing an important role in decreasing this failure. This is because, in order to improve biomaterial performance and hence extend the lifetime of bone implants, it is essential to design surface characteristics that interface optimally with select proteins and subsequently with pertinent bone cell types. That is, immediately after implantation, proteins will adsorb from plasma to biomaterial surfaces to control cell attachment and eventual tissue regeneration (Figure 22.1) [16,17]. Initial protein interactions that mediate cell function depend on many biomaterial properties, including chemistry, charge, wettability, and topography [16,17]. Of significant influence for protein interactions is surface roughness and energy [18–22], and this represents the promise of nanophase materials in bone implant applications.

The critical factor for the merging of nanotechnology with medicine is the increasingly documented, special, biologically improved material properties of nanophase implants compared to conventional formulations of the same material chemistry. This chapter will highlight a novel property of nanophase materials that makes them attractive for use as implants: increased tissue regeneration. Work is ongoing in the domains of orthopedic, dental, bladder, neurological, vascular, cartilage, and cardiovascular applications. However, only orthopedic applications, which are the closest to clinical applications, will be emphasized here. This contribution will briefly articulate the seeming revolutionary changes and the potential gains nanostructured materials can make for bone implant technology.

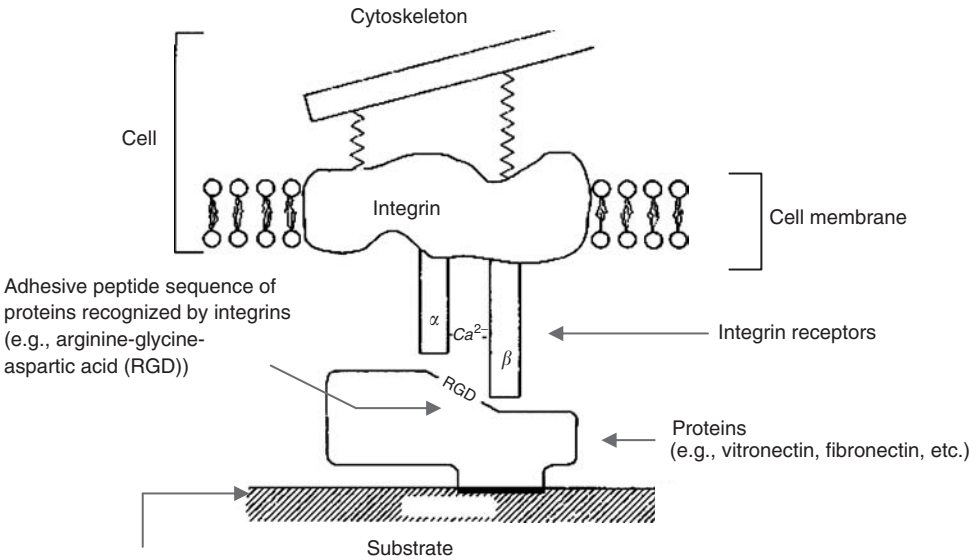

FIGURE 22.1 Cell recognition of biomaterial surfaces controlled by initial protein interactions. Initial protein interactions can influence cell adhesion and, thus, degree of bone tissue formation on biomaterials. Changing material properties will alter protein interactions and influence subsequent cell function. (Adapted and redrawn from Schakenraad, J.M., in *Biomaterials Science: An Introduction to Materials in Medicine*, Ratner, B.D., Hoffman, A.S., Schoen, F.S., and Lemmons, J.E., Eds., Academic Press, New York, 1996, pp. 133–140. With permission.)

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22.2 NANOTECHNOLOGY IN BIOMATERIALS SCIENCE

The emergence of micro- and nanoscale science and engineering has provided new avenues for engineering materials with macromolecular and even down to molecular-scale precision, leading to diagnostic and therapeutic technologies that will revolutionize the way health care is administered. Biomaterials have evolved from off-the-shelf products (e.g., Dacron for vascular grafts) to materials that have been designed with molecular precision to exhibit the desired properties for a specific application, often mimicking biological systems [2,3].

Controlling interactions at the level of natural building blocks, from proteins to cells, facilitates the novel exploration, manipulation, and application of living systems and biological phenomena. Nanostructured tissue scaffolds and biomaterials are being applied for improved tissue design, reconstruction, and reparative medicine [23–26]. Nano- and micro-arrays have been established as the preferred method for carrying out genetic and other biological (e.g., drug discovery) analysis on a massive scale [27]. Natural nanopores [28,29] and synthetic nanopores of tailored dimensions [30,31] are probing, characterizing, and sequencing biological macromolecules and have demonstrated the possibility to analyze the structure of individual macromolecules faster and cheaper [32]. Self-assembly is being applied to create new biomaterials with well-ordered structures at the nanoscale, such as nanofiber peptide and protein scaffolds [33]. In addition, polymer networks with precisely engineered binding sites have been created via molecular imprinting, where functional monomers are preassembled with a target molecule and then the structure is locked with network formation [34].

In medical diagnostics, the speed and precision with which a condition is detected directly impacts the prognosis of a patient. Point-of-care (POC) diagnostic devices, which enable diagnostic testing (*in vivo* or *ex vivo*) at the site of care, can enhance patient outcomes by substantially abbreviated analysis times as a result of the intrinsic advantages of the miniature device and by eliminating the need for sample transport to an on-site or off-site laboratory for testing. The development of micro or miniaturized total analysis systems (μ-TAS), also referred to as lab-on-a-chip devices, has profoundly impacted the corresponding development of POC diagnostic devices. These μ-TAS devices integrate microvalves, micropumps, micro-separations, microsensors, and other components to create miniature systems capable of analysis that typically requires an entire laboratory of instruments. Since being introduced as a novel concept for chemical sensing devices [35], μ-TAS devices have been applied as innovative biological devices [36] and POC diagnostic devices [37,38]. With the further development of micro- and nanosensors, POC diagnostic devices will provide for improved medical management, leading eventually to self-regulated POC diagnostic devices that intermittently or continuously monitor the biological molecule of interest and deliver the therapeutic agents as required.

Additionally, nanoscale science and engineering have accelerated the development of novel drug delivery systems and led to enhanced control over how a given pharmaceutical is administered, helping biological potential to be transformed into medical reality [39]. Micro- and nanoscale devices have been fabricated using integrated circuit processing techniques and have been demonstrated to allow for strict control over the temporal release of the drug. Silicon microchips that can provide controlled release of single or multiple chemical substances on demand via electrochemical dissolution of the thin anode membranes covering microreservoirs have been created [40]. The advantages of this microdevice are that it has a simple release mechanism, very accurate dosing, ability to have complex release patterns, potential for local delivery, and possible biological drug stability enhancement by storing in a microvolume that can be precisely controlled. More recently, multi-pulse drug delivery from a resorbable polymeric microchip device was demonstrated [41].

In particular, the development of polymer systems that are able to interact with their environment in an "intelligent" manner has led to novel materials and applications. These intelligent materials are attractive options as functional components in micro- and nanodevices, due to the ease with which their recognition and actuation properties can be precisely tailored. In this section, neutral and intelligent polymers and networks based on environmentally responsive hydrogels and bio-mimetic polymer networks will be discussed for application as sensing/recognition elements in novel diagnostic

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devices, such as microsensors and microarrays, and therapeutic devices, for tailoring loading and release properties.

In addition to advances in polymer nanotechnology for sensing and recognizing changes in micro-environments, advances have been made concerning tissue regeneration on ceramic and metallic nanomaterials. Broadly speaking, nanotechnology embraces a system whose core of materials is in the range of nanometers (10^{-9} m) [42–52]. The application of nanomaterials for medical diagnosis, treatment of failing organ systems, or prevention and cure of human diseases can generally be referred to as nanomedicine [51,52]. The branch of nanomedicine devoted to the development of biodegradable or nonbiodegradable prostheses fall within the purview of nanobiomedical science and engineering [51,52]. Although various definitions are attached to the word "nanomaterial" by different experts, the commonly accepted concept refers nanomaterials as that material with the basic structural unit in the range 1 to 100 nm (nanostructured), crystalline solids with grain sizes 1 to 100 nm (nanocrystals), individual layer or multilayer surface coatings in the range 1 to 100 nm (nanocoatings), extremely fine powders with an average particle size in the range 1 to 100 nm (nanopowders) and, fibers with a diameter in the range 1 to 100 nm (nanofibers) [42,43].

Since nature itself exists in the nanometer regime, especially tissues in the human body [53], it is clear that nanotechnology can play an integral role in tissue regeneration. Specifically, bone is composed of numerous nanostructures — like collagen and hydroxyapatite (HA) that, most importantly, provide a unique nanostructure for protein and bone cell interactions in the body (Figure 22.2) [50]. Although the ability to mimic constituent components of bone is novel in itself, there are additional reasons to consider nanomaterials for tissue regeneration such as in orthopedic applications: their special surface properties compared to conventional (or micron constituent component structured) materials [47–50]. For example, a nanomaterial has increased numbers of atoms at the surface, grain boundaries or material defects at the surface, surface area, and altered electron distributions compared to conventional materials (Figure 22.3) [50]. In summary, nanophase material surfaces are more reactive than their conventional counterparts. In this light, it is clear that proteins which influence cell interactions that lead to tissue regeneration will be quite different on nanophase compared with conventional implant surfaces (Figure 22.1).

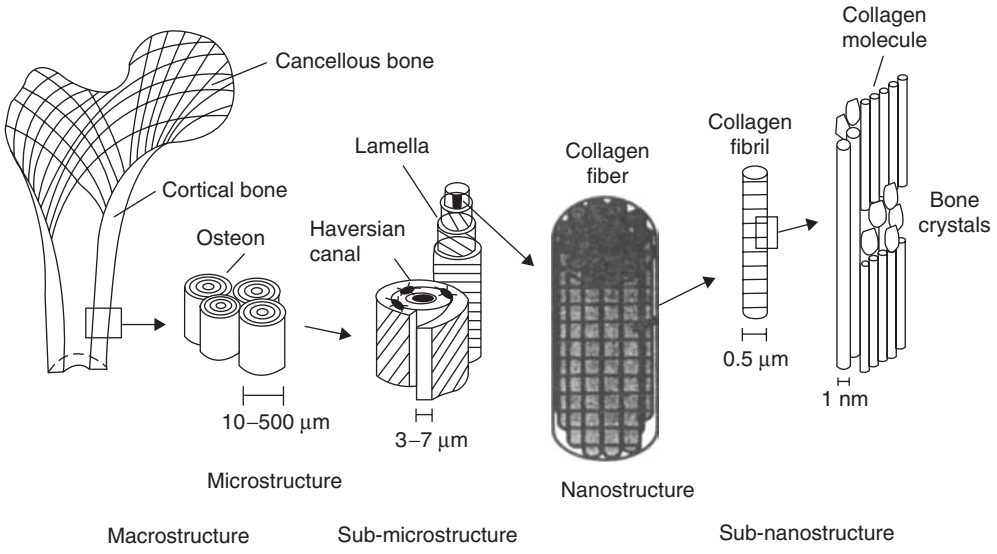

FIGURE 22.2 Nanocomponents of bone provide a high degree of nanostructured surface roughness for bone cells. (Adapted and redrawn from Cowin, R., *Handbook of Bioengineering*, McGraw Hill, New York, 1987. With permission.)

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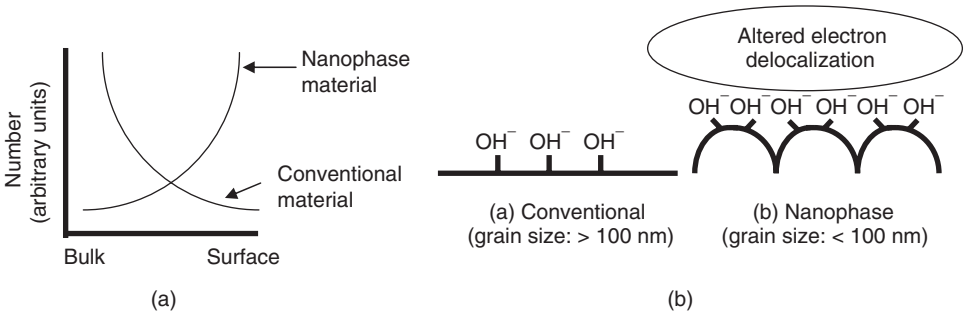

FIGURE 22.3 Special surface properties of nanophase materials. (a) Higher number of atoms at the surface for nanophase compared to conventional materials. (b) Nanophase materials have higher surface areas, possess greater numbers of material defects at the surface, and altered electron delocalization. Such special properties will influence protein interactions for controlling cell functions. (Adapted and redrawn from Klabunde, K.J. et al., *J. Phys. Chem.*, 100, 12141, 1996. With permission.)

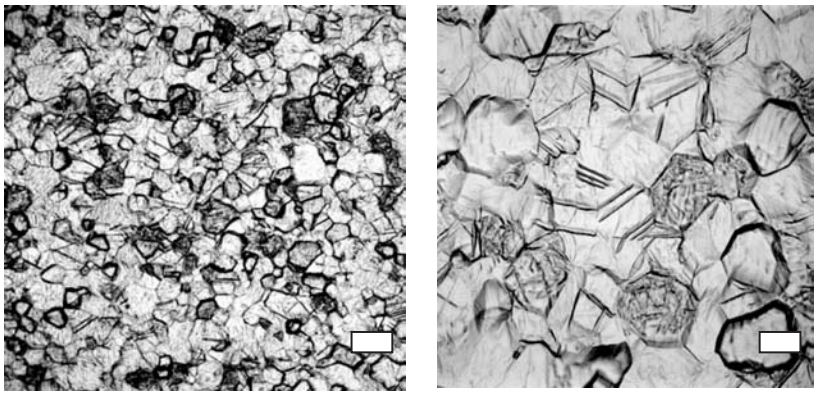

FIGURE 22.4 Conventional grain size of currently used orthopedic implants. Bar = 10 and 1 μm for the left and right micrograph, respectively.

Despite this, the evolution of tissue engineering has centered on the use of materials with non-biologically inspired micron surface features [55,56], mostly changing in chemistry or micron roughness but not degree of nanometer roughness (Figure 22.4). In this context, it should not be surprising why the optimal tissue engineering material (in particular, to regenerate bone) has not been found.

22.3 CURRENT RESEARCH EFFORTS TO IMPROVE BIOMEDICAL PERFORMANCE AT THE NANOSCALE

Nanoscale materials currently being investigated for bone tissue engineering applications can be placed in the following categories: ceramics, metals, polymers, and composites thereof. Each type of material has distinct properties that can be advantageous for specific bone regrowth applications. For example, HA, a ceramic mineral present in bone (Figure 22.2), can also be made synthetically. Ceramics, though, are not mechanically tough enough to be used in bulk for large-scale bone fractures. However, they have found applications for a long time as bioactive coatings due to their ionic bonding mechanisms favorable for osteoblast (or bone-forming cells) function [57].

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Unlike ceramic materials, metals are not found in the body. Due to their mechanical strength and relative inactivity with biological substances, metals (specifically, Ti, Ti6Al4V, and CoCrMo) have been the materials of choice for large bone fractures [55,56]. Polymers exhibit unique properties (such as viscosity, malleability, moldability) and possess mechanical strength that is comparable with many soft (not hard) tissues in the body [54]. To date, because of their excellent friction properties, polymers (like ultra-high-molecular-weight polyethylene) have been primarily used as articulating components of orthopedic joint replacements [58]. Additionally, some polymers (particularly the polyester family) can be resorbed or degraded in the body, which opens the window for controllable repair of damaged bone that is actively being investigated in tissue engineering circles. Lastly, composites of any or all of the above, can be synthesized to provide a wide range of material properties to increase bone implant performance [59]; such ability to tailor composite properties to specific orthopedic applications makes them attractive.

Owing to the numerous materials currently being used and investigated in orthopedics, this review will cover select efforts to create nanoscale surfaces in all of these categories: ceramics, metals, polymers, and composites. Several current and potential materials that have shown promise in nanotechnology for bone biomedical applications as well as needed future directions, will be emphasized.

22.4 SOFT BIOMATERIALS

Polymers are only one of the four major classes of biomaterials; however, polymeric biomaterials have a significant advantage over metals, ceramics, and natural materials in that they can be chemically synthesized or modified according to the desired application. Hydrogels, hydrophilic, and cross-linked polymeric structures, have received considerable attention for use in biomedical applications due to the biocompatible nature of their physical properties [60–62]. Poly(hydroxyethyl methacrylate) [63] is the most widely used hydrogel, and the cross-linked variant has been frequently used in soft contact lenses [64,65]. Hydrogels are also widely used in pharmaceutical applications especially for the oral delivery of therapeutic proteins [66]. This class of materials is also capable of responding to changes in the external pH (Figure 22.5), temperature, ionic strength, nature and composition of the swelling agent, enzymatic or chemical reaction, and electrical or magnetic stimuli [67].

Much consideration must be given when designing a material for a specific application. Certain properties of the material must be controlled so as to perform the necessary function and elicit the

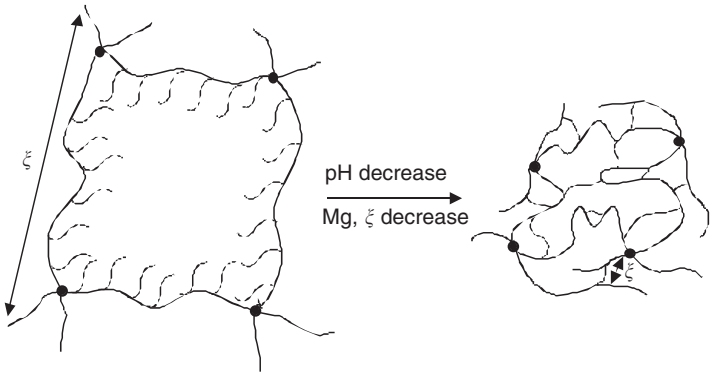

FIGURE 22.5 Network structural changes due to variations on environmental pH. Higher pHs disrupt the interpolymer complexes and ionic moieties deprotonize leading to extensive swelling (left). As pH is decreased, interactions between the tethered grafts with the protonized ionic moieties increase leading to the formation of interpolymer complexes.

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appropriate response. These properties can be tailored to the specific need by carefully controlling structural characteristics, modifying the surface properties, and employing biomimesis in the material design. Hydrogels are a suitable material for biomedical devices because their properties can be controlled during or after the chemical synthesis, providing a significant amount of flexibility to aid in need-specific design applications.

22.4.1 Structural Characteristics

Hydrogel networks are prepared via chemical cross-linking, photopolymerization, or irradiative cross-linking [68] with the behavior of the materials dependent on their equilibrium and dynamic swelling behavior in water. Flory [69] was the first to develop the theory to explain the swelling behavior of cross-linked polymers based upon a Gaussian distribution of the polymer chains. Various parameters have been employed to define the equilibrium-swelling behavior. The volume degree of swelling, Q, is the ratio of the actual volume of a sample in the swollen state divided by its volume in the dry state and q, the weight degree of swelling, is the ratio of the weight of the swollen sample to that of the dry sample [62]. The basic structure of the hydrogel is described by the molecular weight between cross-links, $\overline{M}_c$, and the cross-linking density, ρ_x and can be calculated from Equations (22.1) and (22.2), respectively. The structural characteristics influence the diffusion coefficient of solutes through the network, optical properties, mechanical properties, and surface mobility:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_c} - \frac{\frac{\overline{\upsilon}}{V_1}[1n(1-\upsilon_{2,S})+\upsilon_{2,S}+\chi\upsilon_{2,S}^2]}{\left(\upsilon_{2,S}^{1/3}-\frac{\upsilon_{2,S}}{2}\right)} \tag{22.1}$$

$$\rho_x = \frac{1}{\overline{\upsilon M}_c} \tag{22.2}$$

These key properties of the hydrogel are typically determined during initial synthesis of the biomaterial and are representative of the bulk structure of the material. Advances in nanotechnology have afforded the ability to refine further the structure by molecularly engineering the hydrogel to impart a recognitive capacity. Domains within the molecularly designed hydrogel are able to recognize specific molecules through highly select noncovalent interactions between the building blocks of both the hydrogel network and the recognizable molecule [70].

22.4.2 Surface Properties

In addition to the bulk structural characteristics of the biomaterial, the surface plays a key role in the ability of the material to function as designed. A surface provides a low-energy barrier to mobility, a high accessibility for reaction, enhanced reaction turnover rates, and ultimately allows for molecular recognition [71]. As the first biomaterials were developed (intraocular lenses, hip joint replacements, and blood-contacting devices), the importance of the surface properties, protein–surface interaction, and surface modification was evident to researchers [72,73]. Baier et al. [74] introduced methods with the ability to determine the driving mechanisms behind bioreactions. Hoffman [75] demonstrated that materials could be engineered so as to provide the desired biological responses necessary for effective biomaterial functionality.

In this light, poly(ethylene glycol) (PEG) has received considerable attention for its use at the biomaterial–host interface to prevent protein fouling [76–78]. Castner and Ratner [71] list other strategies in addition to PEG that have been employed in biomaterials to prevent protein adsorption. However, possessing the ability to design surface properties has not been able to fully solve the issues

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associated with the biocompatibility of the material. True biocompatibility will be achieved when absorbing proteins are able to maintain their conformation, eliminating the detrimental foreign body reaction to this newly formed unnatural layer of denatured proteins [71]. The surface of the material must be designed so as to provide components inherent to the natural wound healing process. This is an important contribution nanotechnology can make to improve biomaterial performance.

22.4.3 BIOMIMETICS

The design and development of biomaterials have primarily been focused on the biocompatibility of the device with its surroundings, overlooking the biomolecular interactions that occur. The paradigm for biomaterial characterization has shifted from one focusing on broad design parameters to one that focuses on eliciting specific molecular responses from the physiological environment. Biomimetic materials emulate nature and mimic biological architecture to elicit a desired cellular response [79,126]. Because of their hydrophilic structure, hydrogels inherently possess the characteristics of natural tissue; however, these materials in device form are much larger than individual cells. The major thrust of biomimesis is to design structures that have the ability to interact on a sub-cellular basis.

In designing biomaterials one must understand the mechanisms by which cells interact for efficient biological mimicking. Three specific interactions that are crucial in material development include cell adhesion, morphogenic stimuli signaling, and endocytosis [79]; since all of these are events controlled at the nanoscale, biomimetics is a critical field in nanotechnology.

22.4.4 NANOSCALE BIOPOLYMER CARRIERS

Polymer nanoparticle and nanosphere carriers (Figure 22.6 and Figure 22.7) are very attractive for biomedical and pharmaceutical applications, due to their unique and tailorable properties. In the case of polymer networks, the release profile can be precisely controlled through the design of its molecular structure, such as degree of cross-linking and ionic characteristics of the pendent functional groups [80,81].

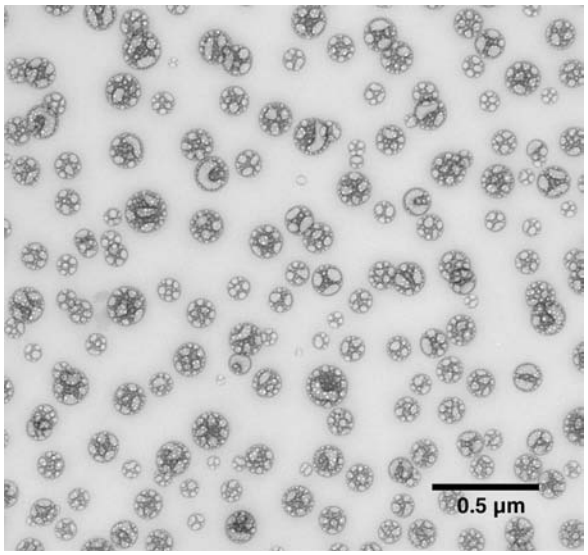

FIGURE 22.6 TEM image of P(MAA-g-EG) nanospheres prepared from a MAA/EG molar feed ratio of 1:1. The P(MAA-g-EG) nanospheres were stained with uranyl acetate at pH 4.0.

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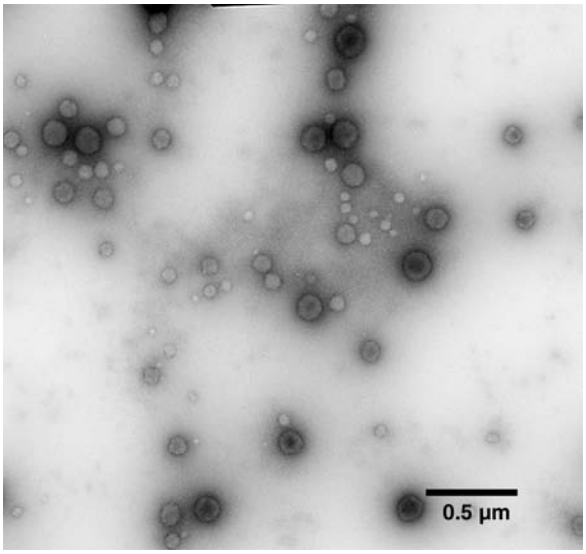

FIGURE 22.7 TEM image of P(MAA-g-EG) nanospheres prepared from a MAA/EG molar feed ratio of 1:1. The P(MAA-g-EG) nanospheres were stained with phosphotugstic acid pH 7.2.

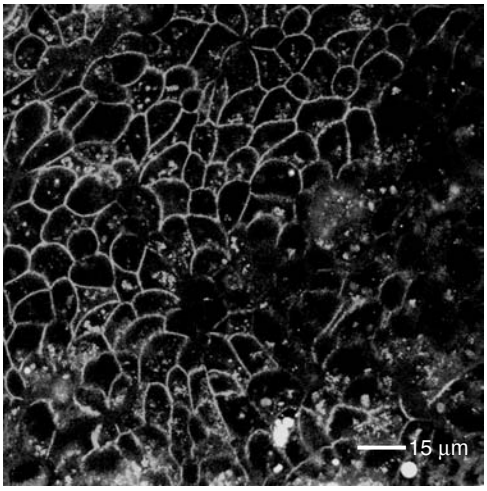

FIGURE 22.8 Optical section of a Caco-2 cell monolayer grown on microporous Transwell® plates obtained with a confocal microscope. FITC-labeled insulin (green) was added to the apical chamber of the cell monolayer in the presence of poly(methacrylic acid-grafted-ethylene glycol) microparticles for 120 min, and images were taken after fixing the cells with 3.7% formaldehyde.

Polymer nanospheres have been molecularly designed to be responsive to the pH of their environment, enabling the protection of fragile therapeutic peptides (Figure 22.8) and proteins in the harsh, acidic stomach environment and then releasing them in the more amiable environment of the upper small intestine [82–84]. In addition, nanoparticle carriers have been designed to have stealth properties, allowing extended residence time without being recognized by the immune system [85,86]. In other efforts, synthetic delivery systems, including polymeric nanoparticles, have been developed for application in gene delivery [87,88]. By creating polymer drug delivery systems that are biodegradable, the need for removal of the system postdelivery is eliminated, since the polymer

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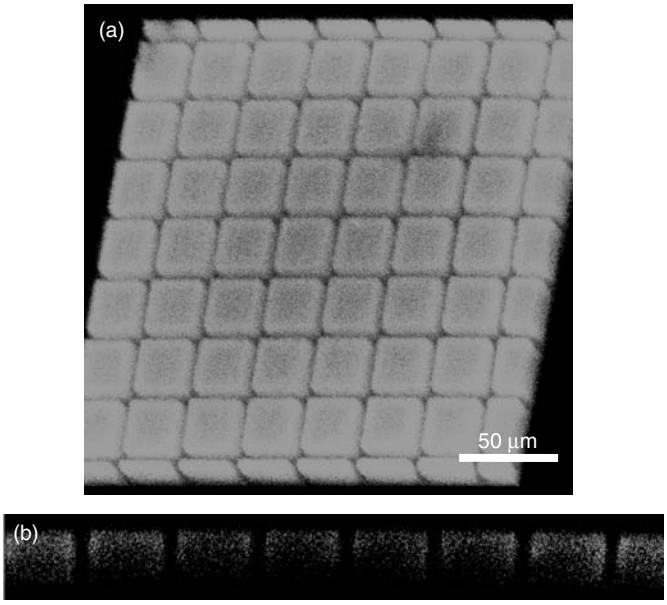

FIGURE 22.9 (a) 3-D projection of a micropatterned square array of a biomimetic polymer network based on a cross-linked polyacrylamide obtained utilizing a confocal microscope. (b) A slice of the square array.

can be naturally resorbed by the body [89]. Also, a number of companies are reformulating insoluble drugs as nanoparticles and nanocrystals to control uptake through cellular membranes.

Microchips (Figure 22.9) have been created for the storage and then delivery of multiple drugs in a controlled manner. For instance, a solid-state silicon microchip that can provide controlled release of single or multiple chemical substances on demand was fabricated and demonstrated [40,90]. The release is achieved via electrochemical dissolution of the thin anode membranes covering the micro-reservoirs filled with chemicals in solid, liquid, or gel form. The advantages of this microdevice include a simple release mechanism, very accurate dosing, ability to have complex release patterns, potential for local delivery, and possible biological drug stability enhancement by storing in a micro-volume that can be precisely controlled. Recently, multi-pulse drug delivery from a resorbable polymeric microchip device was demonstrated [41].

The aforementioned microdevices demonstrate only a few examples of the wide variety of novel applications that exist for integration of micro- and nanofabrication technologies in drug delivery, revealing the immaturity of the field. These novel drug delivery devices can enable efficient delivery that was unattainable with conventional drug delivery techniques, resulting in the enhancement of the therapeutic activity of a drug.

22.5 CERAMIC NANOMATERIALS

Perhaps slightly more mature, is the application of nanophase ceramics in bone tissue engineering applications. The next series of sections will highlight the improvement in bone regeneration that can be obtained through the use of ceramic nanotechnology.

22.5.1 INCREASED OSTEOBLAST FUNCTIONS

The first report correlating increased bone cell function with decreased material grain or particulate size into the nanometer regime dates back to 1998 and involves ceramics [91]. Such reports described how *in vitro* osteoblast (bone-forming cell) adhesion, proliferation, differentiation (as measured by intracellular and extracellular matrix protein synthesis such as alkaline phosphatase), and calcium

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deposition were enhanced on ceramics with particulate or grain sizes less than 100 nm [91–100]. Specifically, this was first demonstrated for a wide range of ceramic chemistries including titania (Figure 22.10), alumina, and HA [93]. For example, four, three, and two times the amount of calcium-mineral deposition was observed when osteoblasts were cultured for up to 28 days on nanophase compared with conventional alumina, titania, and HA, respectively [95]. It is important to note that for each respective nanophase and conventional ceramic mentioned in these first reports, similar chemistry and material phase were studied [91–100]. That is to say, only the degree of nanometer surface features were altered between respective nanophase and conventional alumina, titania, and HA. This is important since as previously discussed it is well known that alterations in surface chemistry will influence bone cell function [12,55–59], but this was the first time changes in the degree of nanometer roughness alone were reported to enhance bone cell responses [91].

Although these studies provided preliminary evidence that osteoblast functions can be promoted on nanostructured materials compared with conventional materials regardless of ceramic chemistry, Elias et al. [101] further described a study where the topography of compacted carbon nanometer fibers were transferred to poly-lactic-glycolic acid (PLGA) using well-established silastic mold techniques. The same procedure was followed for compacts composed of conventional carbon fibers. The successful transfer of nanometer surface features in compacted carbon nanometer fibers and micron surface features in conventional fiber compacts were compared and is illustrated in Figure 22.11 [101]. Importantly, osteoblast adhesion increased on PLGA molds made from nanometer fibers compared to conventional carbon fibers [101]. Increased osteoblast functions were also observed on the original nanometer fiber material compared with conventional carbon fiber compacts [101]. In this manner, this study provided further evidence of the importance of nanometer surface features (and not chemistry) in promoting functions of bone-forming cells.

Equally interesting, a step-function increase in osteoblast performance has been reported at distinct ceramic grain sizes, specifically at alumina and titania spherical grain sizes below 60 nm [93]. This is intriguing since when creating alumina or titania ceramics with average grain sizes below 60 nm, a drastic increase in osteoblast function was observed compared to respective ceramics with grain sizes just 10 nm higher (i.e., those with average grain sizes of 70 nm) [93]. This critical grain size for improving osteoblast function is also of paramount importance since numerous other special properties (such as mechanical, electrical, catalytic) of materials have been reported when grain size is specifically reduced to below 100 nm [42–50]. With this information, evidence has been provided to show for the first time that the ability of nanophase ceramics to promote bone cell function is indeed limited to grain sizes (or subsequent surface features) below 100 nm, specifically below 60 nm [93]. Thus, another novel size-dependent property of nanostructured ceramics has been elucidated by these studies.

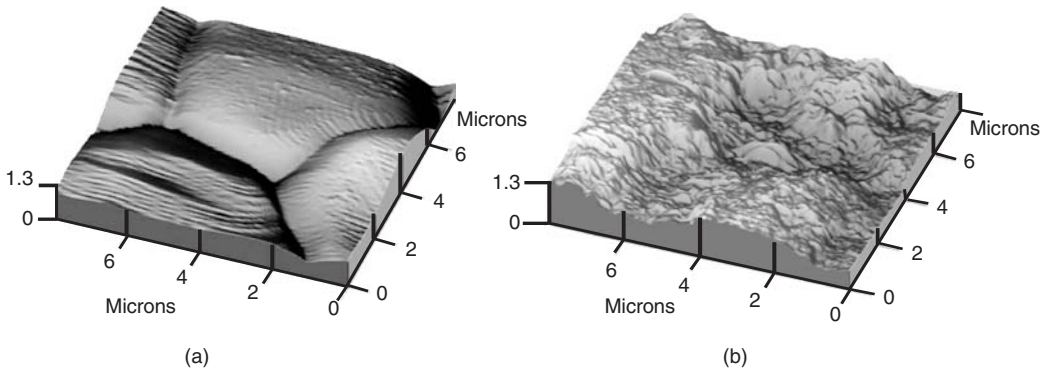

FIGURE 22.10 (a) Conventional and (b) nanophase titania. One of the first studies correlating increased osteoblast function with decreasing ceramic grain size was carried out on titania as pictured here. (From Webster, T.J. et al., *Biomaterials*, 20, 1221, 1999. With permission.)

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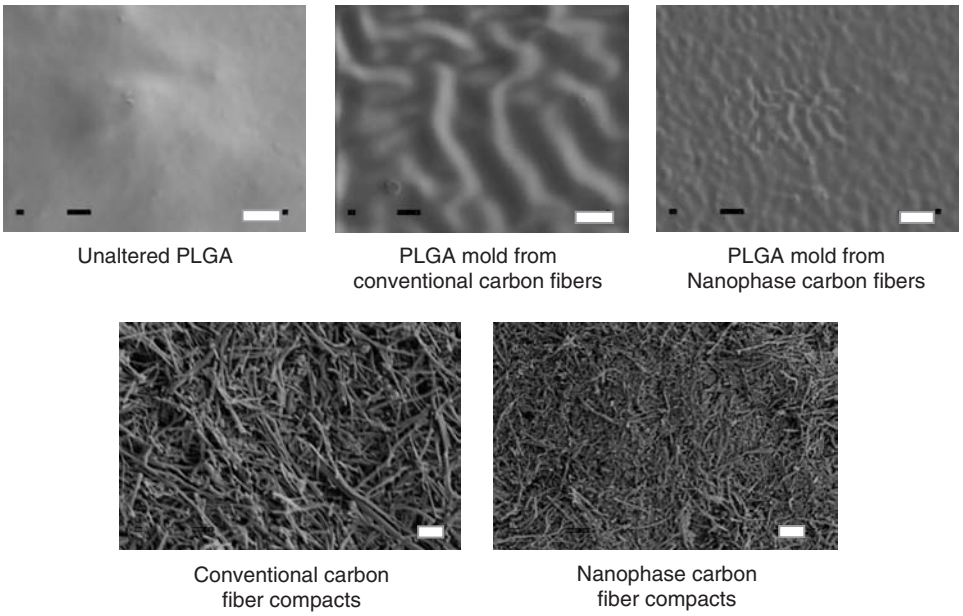
Unaltered PLGA
PLGA mold from conventional carbon fibers
PLGA mold from Nanophase carbon fibers
Conventional carbon fiber compacts
Nanophase carbon fiber compacts

FIGURE 22.11 Poly-lactic-glycolic acid (PLGA) molds of conventional and nanophase carbon fiber compacts. To highlight the importance of nanometer surface roughness regardless of substrate chemistry, studies have shown increased functions of osteoblasts on PLGA molds of nanophase compacts compared to conventional carbon compacts. Studies have also shown increased functions of osteoblasts on compacts composed of nanometer fibers compared to conventional carbon fibers. Bar = 1 μm. (From Elias, K.L. et al., *Biomaterials*, 23, 3279–3287, 2002. With permission.)

Although an exact explanation as to why greater bone regeneration is observed on smaller grain size ceramics in the nanometer regime is not known to date, it is believed that the importance of this specific grain size in improving osteoblast function is connected with interactions of vitronectin (a protein known to mediate osteoblast adhesion with linear dimensions remarkably similar to the critical grain size of 60 nm mentioned above) [94,99]. Moreover, as mentioned previously, several studies have indicated that vitronectin and other proteins important for osteoblast adhesion are more well spread and thus expose amino acid sequences to a greater extent when interacting with nanometer ceramics compared with conventional ceramics [94,99]. It is also intriguing to note that numerous investigators have confirmed that the minimum distance between protein ligands (such as arginine-glycine-aspartic acid or RGD) necessary for cell attachment and spreading is in the nanometer regime (specifically from 10 to 440 nm depending on whether the study was completed with full proteins, protein fragments, or single RGD units) [102–107]. Therefore, an underlying substrate surface that mediates protein spreading (as opposed to protein folding) to expose such ligands, coupled with a nanometer surface roughness to further project such ligands to the cell, may promote cell adhesion due to this optimal ligand spacing.

22.5.2 Increased Osteoclast Functions

In addition to studies highlighting enhanced osteoblast function on nanophase ceramics, increased functions of osteoclasts (bone-resorbing cells) have been reported on nanospherical compared with larger grain size alumina, titania, and HA [100]. Specifically, osteoclast synthesis of tartrate-resistant acid phosphatase (TRAP) and subsequent formation of resorption pits was up to two times greater on nanophase compared to conventional ceramics such as HA. Coordinated functions of osteoblasts and osteoclasts are imperative for the formation and maintenance of healthy new bone

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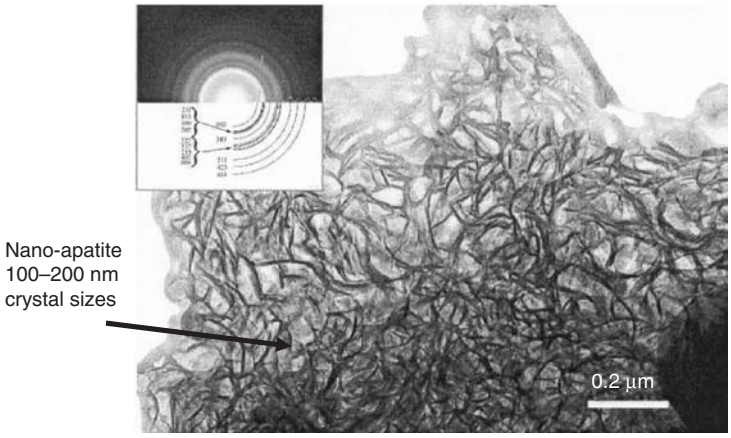
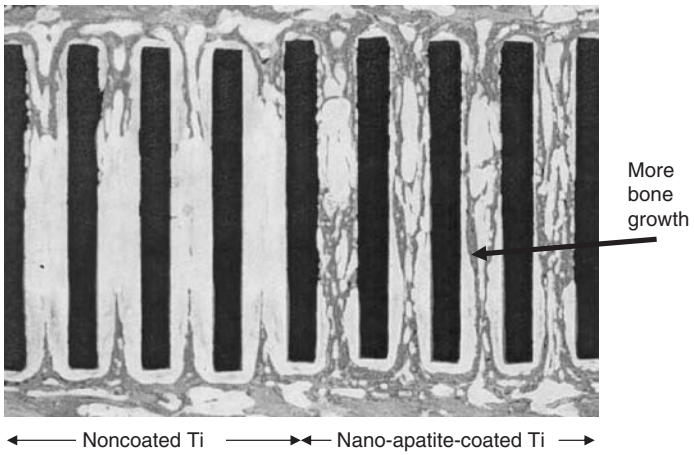
juxtaposed to an orthopedic implant [12]. Frequently, newly formed bone juxtaposed to implants is not remodeled by osteoclasts and thus becomes unhealthy or necrotic [57]. At this time, the exact mechanism of greater functions of osteoclasts on nanophase ceramics is not known, but it may be tied to the well-documented increased solubility properties of nanophase compared with conventional materials [48]. In other words, due to larger numbers of grain boundaries at the surface of smaller grain size materials, increased diffusion of chemicals (such as TRAP) may be occurring to subsequently result in the formation of more resorption pits.

Collectively, results of promoted functions of osteoblasts coupled with greater functions of osteoclasts imply increased formation and maintenance of healthy bone juxtaposed to an implant surface composed of nanophase ceramics. In fact, although not compared with conventional grain size apatite-coated metals, some studies have indeed demonstrated increased new bone formation on metals coated with nanophase apatite [108]. As shown in Figure 22.12, bone formation can be clearly seen on the surface of metals coated with nano-apatite, whereas there is no indication of new bone formation on the underlying metal without the coating [108]. Incidentally, coating metals with nanophase HA has been problematic [109]. For example, owing to their small grain size, techniques



(a)



(b)

FIGURE 22.12 Increased *in vivo* bone regeneration on titanium coated with nanophase apatite. Scanning electron micrograph of nanometer-dimensioned apatite (specifically, between 100–200 nm in size) is depicted in (a). Increased bone regeneration in titanium cages when coated with nano-apatite is depicted in (b). (From Li, P., *J. Biomed. Mater. Res*., 66, 79–85, 2003. With permission.)

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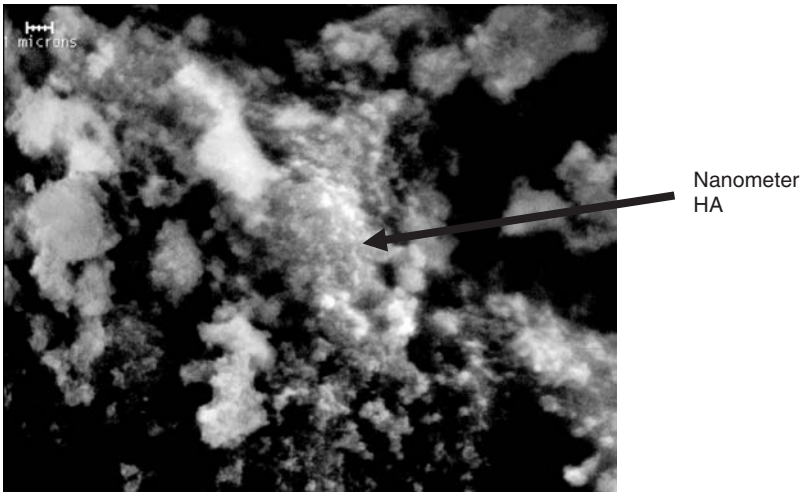

FIGURE 22.13 Nanophase hydroxyapatite coated on titanium. Owing to elevated temperatures, traditional coating techniques, like plasma spray deposition, cannot be used to coat metals with nanophase ceramics. This process developed by Spire Biomedical (Bedford, MA) uses high pressure at low temperatures so as to not allow for grain growth. Bar = 1 μm (upper left).

which use high temperatures (like plasma spray deposition) are not an option since they will result in HA grain growth into the micron regime [109]. To circumvent such difficulties, some investigators have allowed nanophase HA to precipitate on metal surfaces; this can be time consuming and not very controllable [108]. In contrast, others have developed novel techniques which use high-pressure-based processes that do not significantly create elevated temperatures to coat nanophase ceramics on metals so as to retain their bioactive properties (Figure 22.13) [110].

22.5.3 Decreased Competitive Cell Functions

Importantly, it has also been shown that competitive cells do not respond in the same manner to nanophase materials as osteoblasts and osteoclasts do [94,101,111]. In fact, decreased functions of fibroblasts (cells that contribute to fibrous encapsulation and callus formation events that may lead to implant loosening and failure [12] and of endothelial cells [cells that line the vasculature of the body]) have been observed on nanophase compared with conventional ceramics [94]. In fact, the ratio of osteoblast to fibroblast adhesion increased from 1:1 on conventional alumina to 3:1 on nanophase alumina [94].

Previously, such selectivity in bone cell function on materials has only been observed through delicate surface chemistry (e.g., through the immobilization of peptide sequences like Lys-Arg-Ser-Arg or KRSR) [112]. It has been argued that immobilized delicate surface chemistries may be compromised once implanted due to macromolecular interactions that render such epitopes non-functional *in vivo*. For these reasons, it is important to note that studies demonstrating select enhanced osteoblast and osteoclast functions with decreased functions of competitive cells on nanophase materials have been conducted on surfaces that have not been chemically modified by the immobilization of proteins, amino acids, peptides, or other entities [94,101,111]. Rather it is the unmodified, raw material surface that is specifically promoting bone cell functions.

Fibroblast function was also investigated in the same study that was previously mentioned in which Elias et al. transferred the topography of compacted carbon nanometer fibers compared to conventional fibers to PLGA using well-established silastic mold techniques (Figure 22.11) [102]. Similar to the observed greater osteoblast adhesion already noted, decreased fibroblast adhesion was measured on PLGA molds synthesized from carbon nanometer fibers compared to conventional fibers [102]. Again, this was the same trend observed on the starting material of carbon nanometer

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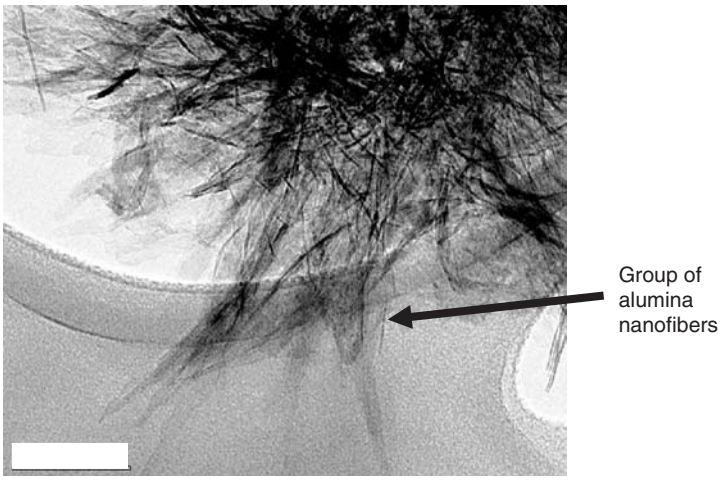

FIGURE 22.14 Transmission electron microscope image of alumina nanofibers. Compared to spherical conventional alumina, increased functions of osteoblasts have been reported on nanophase fibrous alumina. Scale bar = 10 nm. (From Price, R.L. et al., *J. Biomed. Mater. Res.*, 67, 1284, 2003. With permission.)

fiber compacts compared to conventional fiber compacts [102]. Thus, this study demonstrated the importance of a nanometer surface roughness (and not chemical composition of the material) in decreasing functions of fibroblasts that may lead to undesirable fibrous encapsulation and callus formation events inhibiting osseointegration of orthopedic implants with surrounding bone.

22.5.4 Increased Osteoblast Functions on Nanofibrous Materials

Recently, researchers have further modified nanophase ceramics to simulate not only the nanometer dimension but also the aspect ratio of proteins and HA crystals found in the extra-cellular matrix of bone [111]. For example, consolidated substrates formulated from nanofibrous alumina (diameter = 2 nm, length > 50 nm; Figure 22.14) increased osteoblast functions in comparison with similar alumina substrates formulated from the aforementioned nanospherical particles [111]. Specifically, Price et al. [111] determined a twofold increase in osteoblast cell adhesion density on nanofiber vs conventional nanospherical alumina substrates, following only a 2-h culture. Greater subsequent functions leading to new bone synthesis has also been reported on nanofibrous compared to nano- and conventional spherical alumina [111]. Thus, perhaps not only is the nanometer grain size of components of bone important to mimic in materials, but the aspect ratio may also be key to simulate in synthetic materials to optimize bone cell response.

Another class of novel biologically inspired nanofiber materials that have been investigated for orthopedic applications are self-assembled helical rosette nanotubes [113]. These organic compounds are composed of guanine and cytosine DNA pairs that self-assemble when added to water to form unique nanostructures (Figure 22.15). These nanotubes have been reported to be 1.1 nm wide and up to several millimeters wide [113]. Compared to currently used titanium, recent studies have indicated that osteoblast function is increased on titanium coated with helical rosette nanotubes (Figure 22.15) [113]. Although in these studies it has not been possible to separate the influence of nanometer dimensions from the effects of nanotube chemistry on cell functions, it is clear that these nanotubes are another category of novel nanostructured materials that can be used to promote bone formation. It is also intriguing to consider what role self-assembled nanofibers may play in orthopedics since bone itself is a self-assembled collection of nanofibers.

In this context it is important to mention that only nanophase materials can mimic the unique aspect ratio of HA and proteins found in the extracellular matrix of bone; it is not possible for micron-sized

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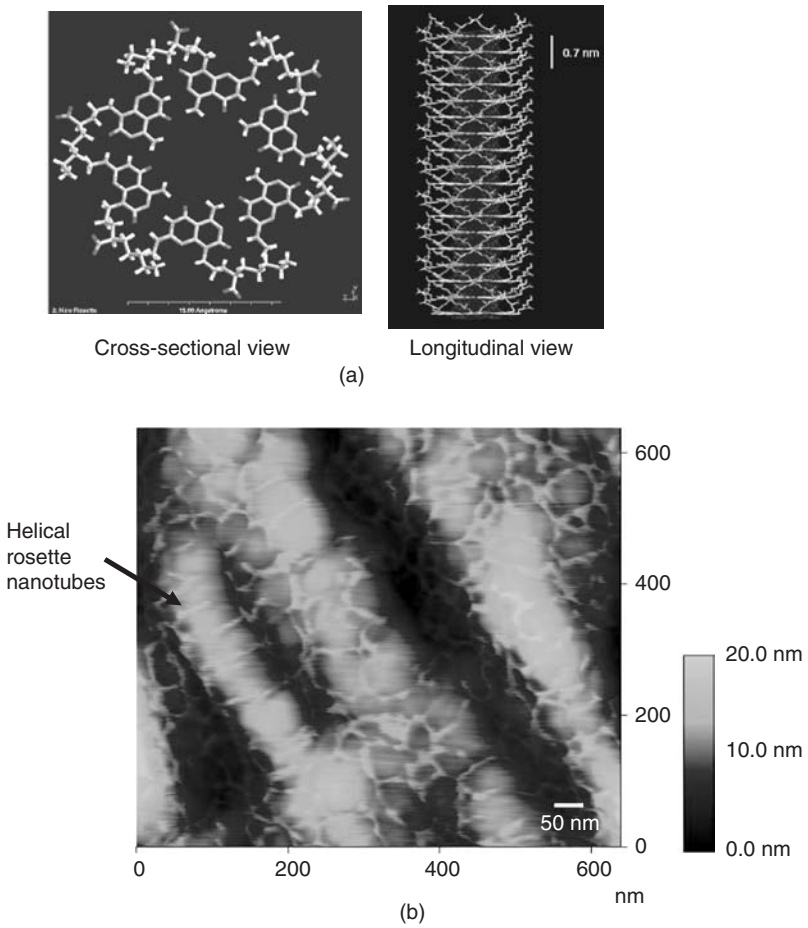

FIGURE 22.15 Helical rosette nanotubes. Drawing of the cross-sectional (left) and longitudinal (right) view of self-assembled helical rosette nanotubes is depicted in (a) while helical rosette nanotubes coated on titanium is depicted in (b). Note the nanophase dimension of these organic tubes. Increased osteoblast function has been observed on helical rosette nanotubes coated on Ti. (From Chun, A. et al., *Nanotechnology*, 15, S234, 2004. With permission.)

materials to simulate the unique nanometer constituent components of bone. As mentioned previously, results concerning the importance of nanofibrous materials in promoting functions of osteoblasts have been reported for carbon and polymer molds of carbon nanofibers (Figure 22.11) [101]. These findings consistently testify to the unprecedented ability to create nanomaterials to mimic the dimensions of components of physiological bone to promote new bone formation.

22.6 METAL NANOMATERIALS

Although much work has been conducted on nanophase ceramics for orthopedic applications to date, several recent studies have focused on the analysis of bone regeneration on nanophase metals. Metals investigated to date include titanium, Ti6Al4V, and CoCrMo [114]. While many have attempted to create nanostructured surface features using chemical etchants (such as HNO_3) on titanium, results concerning increased bone synthesis have been mixed [58]. Moreover, through the use of chemical etchants it is unclear what the cells may be responding to — changes in chemistry or changes in topography. For this reason, as was done for the ceramics in this chapter, it is important

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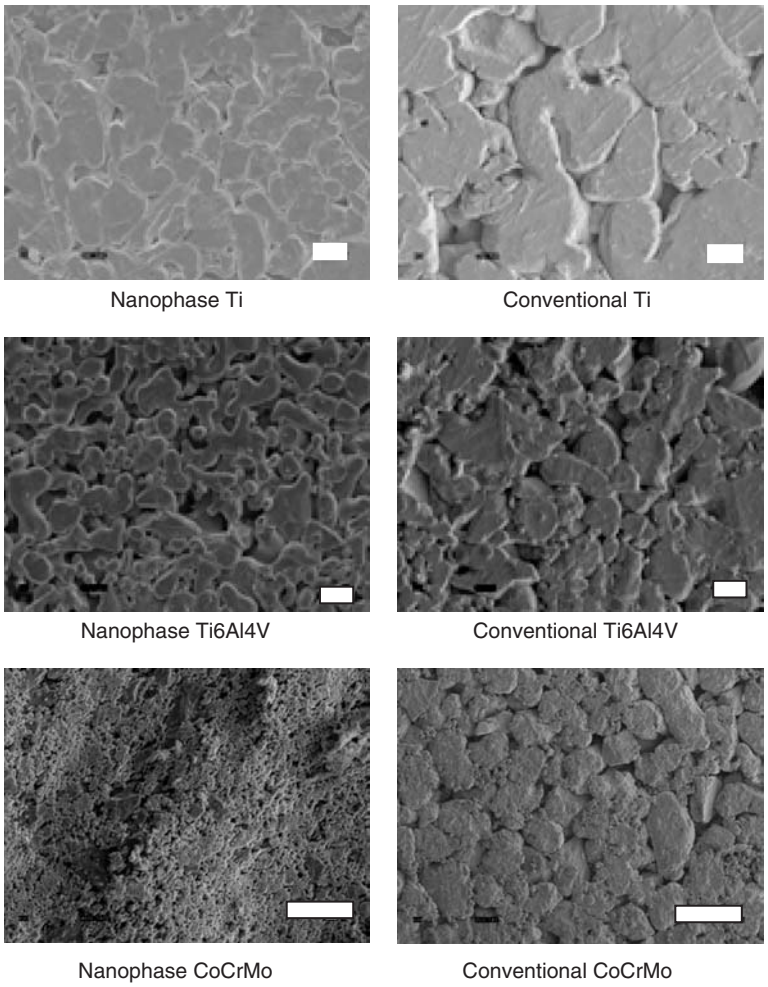

FIGURE 22.16 Scanning electron micrographs of nanophase metals. Increased functions of osteoblasts have been observed on nanophase compared to conventional c.p. Ti, Ti6Al4V, and CoCrMo. Scale bar = 1 μm for nanophase Ti/Ti6Al4V and 10 μm for conventional Ti/Ti6Al4V. Scale bar = 10 μm for nanophase and conventional CoCrMo. (From Ejiofor, J.U. and Webster, T.J., *ASM Conference*, Las Vegas, NV, 2004. With permission.)

to focus on studies that have attempted to minimize large differences in material chemistry and focus only on creating surfaces that alter in their degree of nanometer roughness.

One such study by Ejiofor et al. [114] utilized traditional powder metallurgy techniques without the use of heat to avoid changes in chemistry to fabricate different particle size groups of Ti, Ti6Al4V, and CoCrMo (Figure 22.16). Increased osteoblast adhesion, proliferation, synthesis of extracellular matrix proteins (like alkaline phosphatase and collagen), and deposition of calcium-containing mineral was observed on respective nanophase compared to conventional metals [114]. This was the first study to demonstrate that the novel enhancements in bone regeneration previously seen in ceramics by decreasing grain size can be achieved in metals.

Interestingly, when Ejiofor et al. [114] examined spatial attachment of osteoblasts on the surfaces of nanophase metals, they observed directed osteoblast attachment at metal grain boundaries (Figure 22.17). Because of this, the authors speculated that the increased osteoblast adhesion may be due to more grain boundaries at the surface of nanophase compared to conventional metals. As was the case with nanophase ceramics [94,99], it is plausible that protein adsorption and

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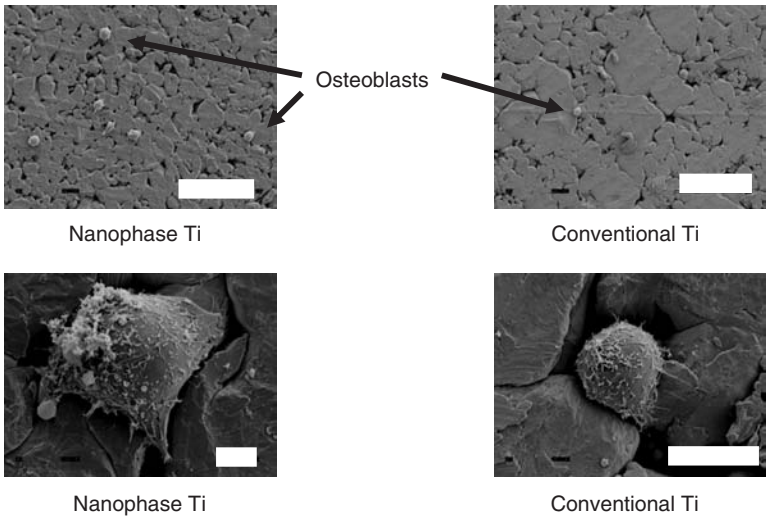

FIGURE 22.17 Scanning electron micrographs of adherent osteoblasts on nanophase c.p. Ti. Directed osteoblast adhesion on nanophase metal grain boundaries has been reported. Scale bar = 100 μm for top and 10 μm for bottom. Adhesion time = 30 min. (From Ejiofor, J.U. and Webster, T.J., *ASM Conference*, Las Vegas, NV, 2004. With permission.)

conformation at nanophase metal grain boundaries may be greatly altered compared to nongrain boundary areas and conventional grain boundaries; in this manner, protein interactions at grain boundaries may be key for osteoblast adhesion.

22.7 POLYMERIC NANOMATERIALS

For ceramics and metals, most studies conducted to date have created desirable nanometer surface features by decreasing the size of constituent components of the material, e.g., a grain, particle, or fiber. However, due to the versatility of polymers, many additional techniques exist to create nanometer surface roughness values. In addition, polymers contribute even further to rehabilitating damaged tissue by possibly providing a degradable scaffold that dissolves within a controllable time while the native tissue reforms. Techniques utilized to fabricate nanometer features on polymers include e-beam lithography, polymer de-mixing, chemical etching, cast-mold techniques, and the use of spin-casting [114–122]. For those that have been applied to bone regeneration, chemical etching followed by mold casting and polymer de-mixing techniques have received the most attention [115,116].

For chemical etching techniques, polymers investigated to date include PLGA (Figure 22.18), PU, and polycaprolactone [116,118–120]. The idea proposed by Kay et al. has been to treat acidic polymers with basic solutions (i.e., NaOH) and basic polymers with acidic solutions (i.e., HNO_3) to create nanosurface features [116]. Kay et al. observed greater osteoblast adhesion on PLGA treated with increasing concentrations and exposure times of NaOH only on two-dimensional films. As expected, data were also provided indicating larger degrees of nanometer surface roughness with increased concentrations and exposure times of NaOH on PLGA. Park et al. [118] took this one step further and fabricated three-dimensional tissue engineering scaffolds by NaOH treatment of PLGA. When comparing osteoblast functions on such scaffolds, even though similar porosity properties existed between nontreated and NaOH-treated PLGA (since similar amounts and sizes of NaCl crystals were used to create the pores through salt-leaching techniques), greater numbers of osteoblasts were counted on and in NaOH-treated PLGA [118]. Unfortunately, due to these fabrication techniques, it is unclear whether the altered PLGA chemistry or nano-etched surface promoted osteoblast adhesion; however, in light

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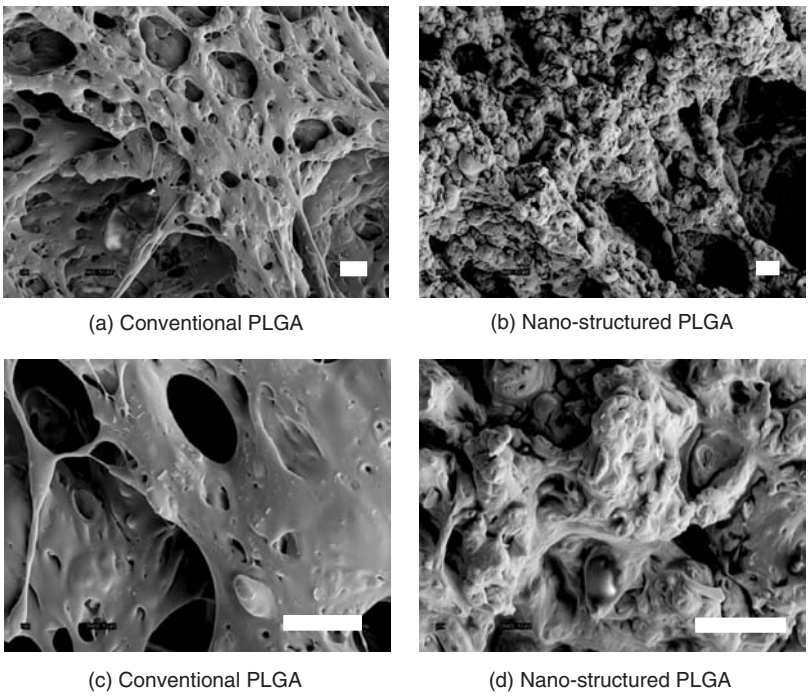

FIGURE 22.18 Scanning electron micrographs of conventional and nanophase PLGA scaffolds. Increased osteoblast functions have been demonstrated on nanophase PLGA scaffolds. Scale bar = 10 μm. (From Park, G.E. et al., *Biomaterials*, 26, 3075–3082, 2005. With permission.)

of the previous studies mentioned in this chapter, the authors of Ref. [118] suggested that the nanometer surface roughness of the NaOH-treated PLGA played an important role [118].

Studies have also been conducted on cell responses to polymers with changes in nanometer surface roughness without changes in chemistry. Specifically, Li et al. utilized polymer de-mixing techniques to create well-controlled nanometer islands of polystyrene and polybromo-styrene [121]. Although osteoblast functions have not been tested on these constructs to date, fibroblast morphology was significantly influenced by incremental nanometer changes in polymer island dimensions (Figure 22.19). Again, this study points to the unprecedented control that can be gained over cell functions by synthesizing materials with nanometer surface features.

Although not related to orthopedic applications, vascular and bladder cell responses have also been promoted by altering the topography of polymeric materials in the nanometer regime [116,118,120,122]. In these studies, chondrocytes [116], bladder [120], and vascular smooth muscle cell [119] adhesion and proliferation were greater on two-dimensional nanometer surfaces of biodegradable polymers such as PLGA, PU, and polycaprolactone; similar trends have recently been reported on three-dimensional PLGA scaffolds [122].

22.8 COMPOSITE NANOMATERIALS

Owing to the previous information of increased osteoblast function on ceramics [100] and polymers [111], bone cell function on nanophase ceramic polymer composites have also been determined. Specifically, studies conducted to date show promoted osteoblast responses on composites of PLGA combined separately with nanophase alumina, titania, and HA (30:70 wt% PLGA/ceramic) (Figure 22.20) [123]. For example, up to three times more osteoblasts adhered to PLGA when it contained nanophase compared to conventional titania particles [116]. Since similar porosity

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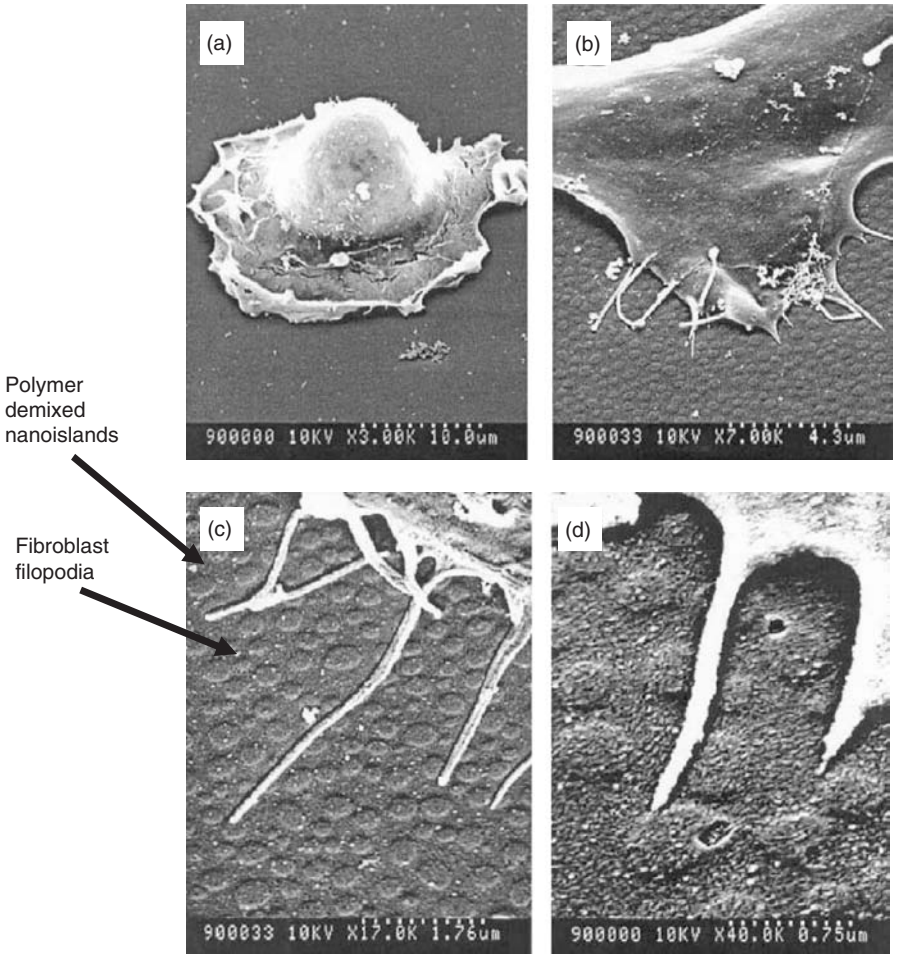

FIGURE 22.19 Polymer nanoislands created by de-mixing polystyrene and polybromo-styrene. Altered cell functions have been observed on polymer nanoislands compared to conventional polymer topographies. (a) through (d) represents increased magnification. (From Dalby, M.J. et al., *Biomaterials*, 23, 2945, 2002. With permission.)

(both percentages and diameters) existed between PLGA with conventional titania compared to nanophase titania, another novel property of nanophase ceramic composites was elucidated in this study: increased osteoblast functions. This is in addition to numerous reports in the literature highlighting greater toughness of nanophase compared to conventional ceramic/polymer composites [44–46].

Moreover, promoted responses of osteoblasts have also been reported when carbon nanofibers were incorporated into polymer composites; specifically, three times the number of osteoblasts adhered on PU with increasing weight percentages of nanometer carbon fibers when compared with conventional dimension carbon fibers (Figure 22.21) [124]. As mentioned, reports in the literature have demonstrated higher osteoblast adhesion on nanophase carbon fibers in comparison with conventional carbon fibers (or titanium [ASTM F-67, Grade 2] [124]), but this study demonstrated greater osteoblast adhesion with only a 2 wt% increase of carbon nanofibers in the PU matrix. Up to three and four times the number of osteoblasts that adhered on the 100:0 PU/CN wt%, adhered on the 90:10 and the 75:25 PU/CN wt% composites, respectively [124]. This exemplifies the unprecedented ability of nanophase materials to increase functions of bone cells whether used alone or in polymer composite form.

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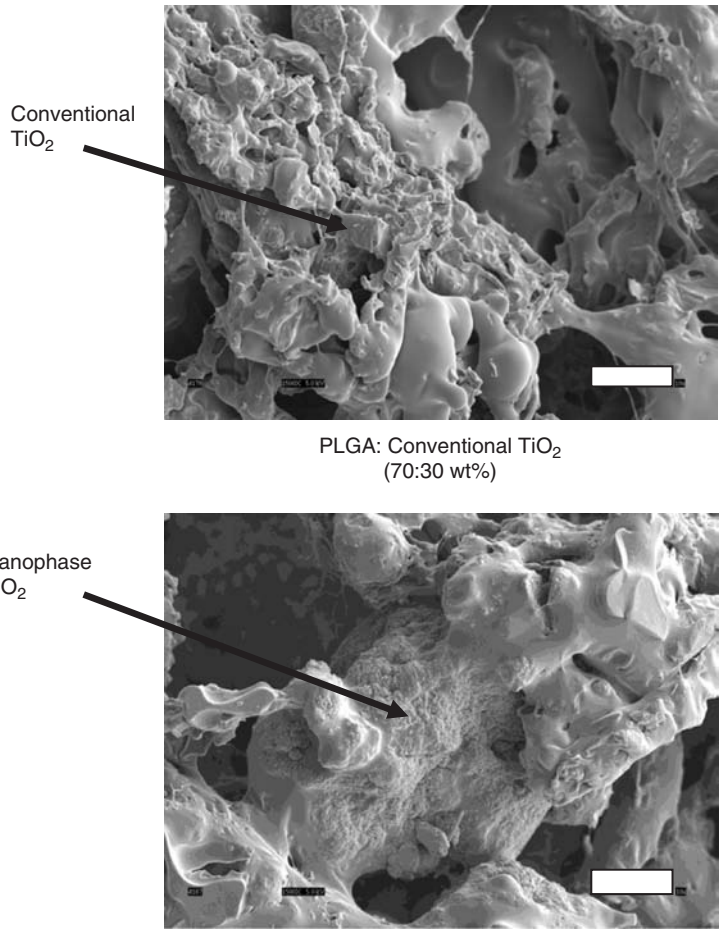

FIGURE 22.20 Scanning electron micrographs of poly-lactic-glycolic acid (PLGA): titania composites. Increased osteoblast function has been observed on polymer composites containing nanophase compared to conventional ceramics. Scale bar = 10 μm. (From Webster, T.J. and Smith, T.A., *J. Biomed. Mater. Res.*, 74, 677–686, 2004. With permission.)

22.9 AREAS OF APPLICATION

While there has already been some effort on incorporating nanotechnology into orthopedic applications, it is clear that this is only the beginning for the incorporation of nanotechnology into biology. In the following sections some additional avenues are highlighted.

22.9.1 DRUG DELIVERY

Polymers have found a significant role in the development of novel drug delivery systems. Biomaterials for muco-adhesive drug delivery applications have been improved through the addition of PEG as an adhesion promoter [126–129]. Additionally, smart hydrogel drug carriers have been molecularly designed to carry safely proteins and peptides to the duodenum region, avoiding the harsh, acidic conditions of the stomach and the proteolytic enzymes present along the gastrointestinal tract [130–132]. Drug delivery systems composed of degradable polymers, such as

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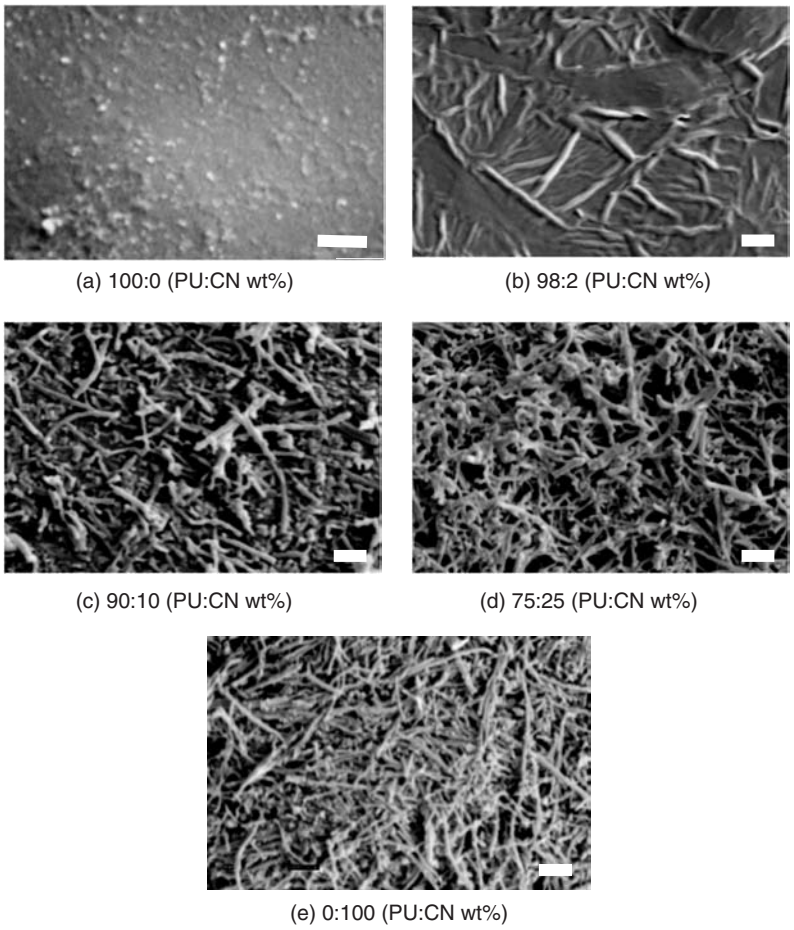

FIGURE 22.21 Scanning electron micrographs of poly-ether-urethane (PU): carbon nanofibers (wt.%) composites. Increased functions of osteoblasts have been observed on polymer composites containing carbon nanofibers. Scale bar = 1 μm. (From Price, R.L. et al., *Biomaterials*, 24, 1877, 2003. With permission.)

polyanhydrides [133–136], polyorthoesters [137,138], and PLGA [139,140], have been implemented in chemically controlled drug delivery.

Lu and Chen [141] have highlighted recent developments in the nanofabrication of biodegradable drug delivery systems, and Leoni and Desai [142] have offered a review of the abilities to create nanoporous structures containing an optimal pore size and distribution. Nanoporous structures [143] have the ability to allow mass transport of desirable compounds but limit those that are undesirable. An example given in the aforementioned review is the nanoporous encapsulation of pancreatic islet cells. The controlled pore size could allow certain molecules such as glucose transport but significantly hinder the diffusion of molecules responsible for causing immune response-mediated device failure such as immunoglobulin G and M molecules (IgG and IgM).

Nanodrug delivery systems are quickly evolving in their ability to integrate biologically complex components into a functional nanodevice. Lee et al. [144] offer the bacmid process as an object-oriented approach for designing novel nanosystems and outline the possibility of using this type of design process for effective delivery of vaccines. Santini et al. [40] have demonstrated the ability to deliver nanoliter quantities of therapeutics on demand from an array of sealed reservoirs. Drug discovery and delivery are becoming sciences that encompass skills in nanotechnology,

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microtechnology, and biology to design systems effectively more capable of achieving efficient and effective therapeutic treatments [145].

Much effort has been dedicated to engineering nanoparticulate drug delivery systems [146,147]. Surface modification allows the specific targeting of particles and enhances their ability to interact with certain types of cells [148]. Size plays a key role in the ability of particles to participate in intracellular uptake, and biodegradable nanoparticles can be used as sustained-release delivery systems once inside the cytoplasm [149].

22.9.2 Tissue Engineering

Tissue engineering strives to create living tissue and organs through the use of synthetic, hybrid, or natural materials that have been designed or fabricated in a way to elicit a desirable cellular response from the scaffold [150]. The major thrust of developing materials for tissue engineering is providing the cells an environment in which they can continue their normal functionality. Biodegradable and resorbable materials are favorable due to the lack of necessity of a material structure to be present once the matrix has been formed. Poly(lactic acid), poly(glycolic acid), and their copolymers (PLGA) were first utilized in the development of biodegradable materials for tissue engineering applications, followed by other types of materials that include PUs [151], polyanhydrides [152,153], and, more recently, poly(ether anhydrides) [154].

This chapter has provided some nanotechnology-based examples of tissue engineering advances in orthopedics. In addition, Tsang and Bhatia [155] offer an extensive review into the fabrication techniques developed to aid in the development of novel tissue scaffolds. Significant advances in fabrication using heat, light, adhesives, and molding are elaborated upon. The use of cells to self-assemble native cellular matrices and cell/scaffold hybrids are also highlighted. Biomimetic materials are actively being pursued as integral components of novel tissue engineering biomaterials [156,157]. Various techniques are being employed to take advantage of the ability to modify the surface of tissue engineering materials, with RGD modification receiving the most attention [158].

Synthetic hydrogels have traditionally been employed in the development of tissue engineering scaffolds due to their high biocompatibility, hydrophilicity, and tissue-like properties. The molecular design of these materials affords the engineer the ability to impart certain physical properties into the device to obtain the necessary physiological response. Poly(lactic acid)-g-poly(vinyl alchol) (PLA-g-PVA) have been developed for heart valves [159], and PVA/poly(vinyl pyrrolidone) (PVA/PVP) blends have been proposed for nucleus pulposus replacement [160]. Natural materials that have been developed for tissue engineering applications include collagen, hyaluronic acid, alginate, and chitosan [161].

Hybrid materials are being utilized to take advantage of the ability to synthesize polymeric materials with specific properties combined with a bioactive entity that helps elicit a particular biological response. Chondroitin sulfate, a biological polymer and PVA, a synthetic polymer, have been used to synthesize hydrogels that promote chondrogenesis [162]. Mixtures of peptides and synthetic polymers are combined in order to mimic extracellular matrix proteins to enable natural wound healing and reduce the formation of fibrous encapsulation. RGD has been used frequently to impart these properties into a biomaterial [158].

22.9.3 Biological Micro-Electro-Mechanical Systems

Most research focusing on biological micro-electro-mechanical systems (BioMEMS) is for their use in diagnostic devices and for the detection of DNA, viruses, proteins, and other biologically derived molecules [163]. Nanoscale BioMEMS could allow for the real-time detection and analysis of signaling pathways, which would further our knowledge and understanding of the basic mechanisms and functions of the cell. While nanoscale BioMEMS is at its infancy, it is clear that nanotechnology will play an important role in its development.

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22.10 CONSIDERATIONS AND FUTURE DIRECTIONS

Although preliminary attempts to incorporate nanotechnology into biomedical applications seem promising, numerous urgent questions still remain with regard to this new field. First and foremost, the question of safety of nanoparticles once in the human body remains largely unanswered both from a manufacturing point of view and when used in full or as a components of an implantable device. Since such particles are smaller than many pores of biological tissues, it is clear that this information will have to be obtained before further consideration of implantable nanomaterials is undertaken. Such nanoparticles can easily become dislodged from implants during surgical implantation or by fragmentation of articulating components of a joint prosthetic composed of nanophase materials. Although preliminary *in vitro* studies highlight a less adverse influence of nanometer compared to micron particulate wear debris on bone cell viability [164,165], many more experiments are needed, especially *in vivo* to evaluate their efficacy.

Specifically for orthopedic applications, additional questions remain. For example, once exact optimal nanometer surface features are elucidated for increasing bone regeneration, inexpensive tools that can be used in industry will be required. In this context, if the only nanofabrication devices that can be used to synthesize desirable nanometer surface features for bone regeneration are e-beam lithography or other equally expensive techniques, industry may not participate in this boom of nanotechnology at the intersection of tissue engineering. Inexpensive but effective nanometer synthesis techniques must continually be a focus of many investigators.

The direction of the nanotechnology should be and is geared toward dealing with these issues. For example, according to the U.S. government's research agenda, current and future broad interests in nanobiomedical activity can be categorized into three broad related fronts [51,52]:

1. development of pharmaceuticals for inside-the-body applications — such as drugs for anti-cancer and gene therapy
2. development of diagnostic sensors and lab-on-a-chip techniques for outside-the-body applications — such as biosensors to identify bacteriological infections in biowarfare
3. development of prostheses and implants for inside-the-body uses

Whereas the European governments emphasize commercial applications in all the three fronts mentioned above, according to Marsch [52], the U.S. government tends to lean toward fundamental research on biomedical implants and biodefense, leaving commercial applications to industry. Both classifications identify nanophase biomedical implants as a potential interest. The biological and biomimetic nanostructures to be used as orthopedic implants involve some sort of an assembly in which smaller materials later on assume the shape of a body part, such as hip bone. These final biomimetic, bulk nanostructures can start with a predefined nanochemical (like an array of large reactive molecules attached to a surface) or nanophysical (like a small crystal) structure. It is believed that by using these fundamental nanostructured building blocks as seed molecules or crystals, a larger bulk material will self-assemble or keep growing by itself.

In summary, it is now believed that significant evidence exists that highlights the promise nanotechnology has for biological applications, particularly in the bone arena. Clearly, nanomaterials as mentioned here are at their infancy and require much more testing before their full potential can be realized. However, even if nanophase materials never make it to the marketplace due to safety concerns, we have already learned much about how cells interact with surfaces through their application in the orthopedic environment.

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